Chromosome counts in the *Mentha* collection at the USDA–ARS National Clonal Germplasm Repository

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Summary

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The present chromosome number survey is based on the holdings of the National Clonal Germplasm Repository in Corvallis, which houses a world *Mentha* collection which includes most of the known taxa. The survey complements previously published counts and provides information about 73 accessions that are available to researchers. It includes chromosome counts for two accessions of *M. australis,* a previously uncounted Australian endemic, and accessions of *M. japonica, M. diemenica,* and *M. cunninghamii,* taxa with but one or a few published previous counts.

Introduction

Mentha is a genus of wide distribution and considerable economic importance. Shoots and leaves of several species are often used as a condiment. The essential oils, which are steam distilled from the herbage, are processed into flavourings for food, medicine, mouthwash, toothpaste and powder, chewing gum, and candy.

In modern taxonomic treatments of the genus (Harley & Brighton, 1977), there are five sections *(Mentha* sect. *Audibertia,* sect. *Eriodontes,* sect. *Mentha,* sect. *Preslia,* and sect. *Pulegium)* containing 19 species and 13 named hybrids involving species of *M.* sect. *Mentha.* However, over 2300 names have been published (Tucker & al., 1980), and this has created great confusion in the literature. Harley (1967, 1975), Harley & Brighton (1977), Tucker & al. (1980), Tucker & Fairbrothers (1990), and Tucker & al. (1991) have helped resolve many of the taxonomic and nomenclatural problems. A major contribution to the cytology of the genus was the work of Harley & Brighton (1977) which listed chromosome counts of many accessions of almost all the taxa recognized today, with major emphasis on *M.* sect. *Mentha.*

Since 1977, new chromosome counts, confirmation of previous counts, and a few unexpected chromosome numbers have been reported (Humphries & al., 1978; Diana-Corrias, 1980; Gill, 1981; Löve & Löve, 1982; Fagbemi & Morton, 1982; Markova, 1983; Astanova, 1984; Gill, 1984; Silvestre, 1984; Pogan & al., 1986; Sokolovskaya & al., 1986; Tyagi & Naqvi, 1987; Panetta, 1986; Tucker & Fairbrothers, 1990; and Chambers, 1991).

Tyagi & Naqvi (1987) correlated chromosome numbers with yield and quality of oil in six clones of *Mentha arvensis* var. *piperascens* Malinv. ex Bailey. All were from Japan originally, but three had recently been introduced into India from Thailand, Taiwan and Brazil. Two were bud sports from the Thailand clone. The somatic chromosomes ranged from 72 to 108, and the oil content, yield and major components varied widely.

In 1983, a *Mentha* collection, numbering some 480 accessions, was deposited at the National Clonal Germplasm Repository (NCGR) in Corvallis, Oregon (U.S.A.).

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This collection was initially acquired from Merritt J. Murray, plant breeder with the A. M. Todd Company, Kalamazoo, Michigan. Murray collected *Mentha* species, varieties, and cultivars from Europe and North America and received seed and clonal starts from researchers and others all over the world. The collection contained accessions from Baquar (Pakistan), Harley and Stuart (England), Hegnauer (The Netherlands), Thomas, Tucker, Stevenson (U.S.A.), Fester (Argentina), Santos (Brazil), Sobti (India), and Rudloff (Canada). Murray also provided plant material to researchers for chromosomal, chemical, biochemical and genetic studies from the 1950's until 1972, at which time he gave his collection to C. E. Horner, USDA plant pathologist at Oregon State University. Donald D. Roberts maintained and added to the collection during his tenure as USDA mint breeder in the early 1980's. Many duplicates and plants of uncertain parentage were discarded before and after the NCGR received the collection. Since 1983 the repository has acquired additional germplasm, and the collection currently includes approximately 500 accessions of 42 taxa and 130 artificial interspecific hybrids (Chambers, 1992; Chambers & Hummer, 1992). Many of the clones that Murray and others used in earlier studies are currently available. Murray's old accession numbers are on record and are available to researchers from the repository, along with cuttings or *in vitro* cultures, by request from the repository.

A chromosome survey of the *Mentha* collection at NCGR was initated in 1988. One of the goals of the current study was to obtain counts for unreported taxa, those that had few published counts, and those with differing published chromosome numbers. A second goal was to obtain at least one chromosome count for each taxon in the repository collection.

Material and methods

Most chromosome counts were obtained from dividing pollen mother cells (PMC) in flower-buds, or from meristematic cells in root-tips. Clusters of flower buds from plants grown in the greenhouse or screenhouse at NCGR were fixed in modified Carnoy's solution composed of 6 parts chloroform : 3 parts 95 % ethanol : 1 part glacial acetic acid. After 48 hours the buds were placed in 70 % ethanol for 24-48 hours then in fresh 70 % ethanol before staining or storage in a freezer. Young root-tips (approximately 1 cm long) were excised from stem pieces that had been placed in a moist chamber and pretreated with saturated monobromonaphthalene, paradichlorobenzene, or tap water for 4-7 hours at 2-4°C.

Tissues were stained in Snow's alcoholic carmine at 50-60°C for 3-7 days, and then rinsed in 70 % ethanol. Roots were placed in 45 % acetic acid on a slide and hydrolysed on an embedding tray for 5 minutes before the addition of Hoyer's mounting media. Excised anthers were heated for 30 seconds on the embedding tray after the cover slip was in place. Tissues were tapped and squashed to spread and flatten the dividing cells.

Drawings of chromosomes were made from tracings of photographic negatives while consulting prints of photographs made at different focal planes and while viewing the cells under the light microscope at 1250× magnification. The chromosomes were inked in and enlarged by xerography before final drawings were made. Cells were measured using a calibrated ocular micrometer, and the appropriate scale placed on the drawings before enlargement or reduction.

The origin (country, state, county, city, etc.), if known, and whether from cultivation *(ex horto),* is given for each accession that has been counted. The chromosome data are linked to the *Mentha* (MEN) accession numbers in a permanent collection at the National Clonal Germplasm Repository, Corvallis. More complete collection information may be available upon request (exact locality, field observations, collector, date). Voucher specimens of *M. australis, M. cunninghamii, M. diemenica* and *M. japonica* used for chromosome counts have been placed in the Oregon State University herbarium (OSC).

Results and discussion

Table 1 presents the new counts, for plants from areas of the natural or introduced range of the taxa or from cultivation. The species are arranged by sections in the system of Bentham (1848) as modified by Harley & Brighton (1977). Hybrids between species of *Mentha* sect. *Mentha* are listed alphabetically. The accessions that were part of the Murray collection are indicated with an asterisk.

Mentha sect. *Audibertia* ($x = 9$). The count for *M. requienii,* $2n = 18$, agrees with earlier published counts (Harley & Brighton, 1977; Morton in Lawrence, 1978; Diana-Corrias, 1980). This species, the only one with $x = 9$, shares very few morphological traits with other European species but many with *M. cunninghamii,* the endemic species of New Zealand.

Mentha sect. *Eriodontes* ($x = 5?, 10?, 12?$). Kokkini (1992) refers to this section as a catch-all group containing species with unclear relationships. *M. australis,* one of the most widespread of the Australian endemic species, has $2n = 72$ in two clones, one from Victoria and one from New South Wales. From the former, a dividing PMC at metaphase$_{II}$, with 36 chromosomes clearly countable in one group, is shown in Fig. 1.

Our counts of *Mentha cunninghamii* collected in the wild on North Island, New Zealand, was $2n = 72$ (36 bivalents; Fig. 2), agreeing with an earlier published count from South Island plants (Hair & Beuzenberg, 1960).

Our count ($n = 60$) for *Mentha diemenica,* shown in Fig. 3, is a second meiotic metaphase. It does not agree with an earlier count of $2n = 140$ (Morton in Lawrence, 1978).

Our count $2n = 50$ for *Mentha japonica* differs from earlier counts of $2n = 49$ by Nagao (1941) and $2n = 48$ by Ikeda & Udo (1954), Ikeda & Ono (1967) and Ikeda & al. (1970). Shimizu & al. (1962), in a more detailed study of *M. japonica* and its hybrids, also reported $2n = 48$. Many PMCs were countable in our material. Metaphase$_{I}$ had 25 bivalents visible in end view, and metaphase$_{II}$ consistently had 25 chromosomes on both metaphase plates, as is shown in Fig. 4.

Mentha sect. *Mentha* (incl. sect. *Verticillatae* Benth.) ($x = 12$). We here report a count of $2n = 96$ in an accession of *M. aquatica* from the Netherlands

Previously published counts for *Mentha arvensis,* commonly called corn mint, show a pattern that reflects the geographical origin of the plants. Whereas most North American and Asian collections are octoploid ($2n = 96$), those from Europe, with few exceptions, are hexaploid ($2n = 72$). Our accession of *M. arvensis* from the Netherlands has been $2n = 72$. The exceptions include the Pogan & al. (1986) report of $2n = 36$

Table 1. Chromosome counts in *Mentha:* somatic numbers based on NCGR accessions. Asterisked (*) accessions are part of the M. J. Murray collection. P.I. numbers are from the USDA Plant Introduction division. More complete collection data, as available, are supplied with requested plant material.

Taxon	Accession No.	2*n*	Origin
Mentha sect. *Audibertia* (Benth.) Briq.			
– *requienii* Benth.	1*	18	England (seed)
M. sect. *Eriodontes* Benth.			
– *australis* R. Br.	643	72	Australia: Victoria
	690	72	Australia: N.S.W., Wanaaring (seed from herbarium specimen)
– *cunninghamii* Benth.	666	72	New Zealand: North Isl., Turakina
– *diemenica* Spreng.	667	120	Australia: N.S.W., near Wooli
– *japonica* (Miq.) Makino	578	50	Japan: Honshu, Ibara Ki-Ken
M. sect. *Mentha*			
– *aquatica* L.	566	96	Netherlands: Rockanje
– *arvensis* L. var. *arvensis*	567	72	Netherlands: Haarlem
– – var. *canadensis* L.	157*	96	USA: Pennsylvania, Lehigh County
	159*	96	Canada: Ontario
	557	96	U.S.A.: Pennsylvania, Allentown
	565	96	U.S.A.: Oregon, Wheeler County
	571	96	U.S.A.: Oregon, Linn County
	572	96	U.S.A.: Oregon, Deschutes County
– *longifolia* (L.) Huds. subsp. *longifolia*	18*	24	Netherlands: Dordrecht
	19*	24	France: Dijon
	500	48	Afghanistan: Kataghan (P.I. 212313)
	501	48	Afghanistan: Herat (P.I. 212314)
	592	24	Uzbekistan: Tashkent
– – subsp. *polyadena* (Briq.) Briq.	584	24	South Africa
– – subsp. *capensis* (Thunb.) Briq.	585	24	South Africa
– – subsp. *hymalaiensis* Briq.	635	24	USA: Washington, DC. *(ex horto)*
– *spicata* L.,			
– – rugose-leaf form (*M. cordifolia* auct.)	50*	48	USA: Washington
	51*	48	Australia: S. A., Adelaide *(ex horto)*
	52*	48	Australia: S. A., Adelaide *(ex horto)*
	53*	48	USA: New Mexico, Santa Fe
	55*	48	England *(ex horto)*
	363	48	USA: California, Yuba City
	586	48	Brazil: São Paulo
– – 'Native Spearmint'	32*	36	USA: Florida, St. Cloud
– – glabrous-leaf form	27*	48	Australia: S.A., Adelaide *(ex horto)*
	70*	48	Italy: Trieste *(ex horto)*
	72*	48	Italy: Trieste *(ex horto)*
	73*	48	Netherlands: Leiden *(ex horto)*
	86*	48	Netherlands: Amsterdam *(ex horto)*
	90*	48	U.S.A.: Pennsylvania, Easton

Table 1. continued

Taxon	Accession No.	2*n*	Origin
	97*	48	England: Kew (*ex horto,* S_1 high menthone strain)
	625	48	U.S.A.: Oregon, Linn County *(ex horto)*
– – hairy-leaf form	95*	48	U.S.A.: Maine, Camden *(ex horto,* as 'Silver Mint', i.e., *M. longifolia)*
	105*	48	Turkey: Istanbul (as *M. tomentosa*)
– – crisp-leaf form	64*	48	Brazil: Campinas
	104*	48	England: Leeds
	591	48	Tajikistan: Dushanbe
– *suaveolens* Ehrh. subsp. *suaveolens*	8*	24	France: Colmar
	10*	24	Netherlands
	13*	24	Bulgaria: Sofia
	379	24	U.S.A.: Minnesota, Minneapolis (*ex horto,* as 'Curled Mint')
	545	24	Portugal: Lisbon *(ex horto)*
– – 'Variegata'	587	24	U.S.A.: Oregon, Marion Co. *(ex horto)*
– – subsp. *insularis* (Req.) Greuter	574	24	France: Antibes (*ex horto,* I.N.R.A.)
– ×*rotundifolia* (L.) Huds.	179*	24	U.S.A.: California, Berkeley *(ex horto)*
	573	24	France: Antibes *(ex horto)*
– ×*villosa* subsp. *alopecuroides* (Hull) Briq.	45*	36	U.S.A.: New Jersey, Gladstone *(ex horto)*
	400*	36	U.S.A.: Oregon
	403*	36	U.S.A.: New Jersey, New Brunswick *(ex horto)*
M. sect. *Preslia* (Opiz) Harley			
– *cervina* L.	5*	36	Europe
	526	36	Hungary *(ex horto)*
	527	36	Germany *(ex horto)*
M. sect. *Pulegium* (Mill.) DC.			
– *gattefossei* Maire	4*	40	Morocco (seeds received from England)
	530	48	Austria: Hohenleiten *(ex horto)*
	546	48	Germany: Mainz *(ex horto)*
– *pulegium* L.	2*	20	U.S.A.: Oregon, Benton County
	626	20	U.S.A.: Oregon, Benton County
	549	30	U.S.A.: Oregon, Lane County
	577	40	U.S.A.: Oregon, Linn County
	589	20	England *(ex horto)*
	3*	30	Poland
	489	20	England: Folkstone (*ex horto,* U.S.A.)
	502	20	Tunisia: Ariana (*ex horto,* P.I. 196272)
	505	20	Tunisia: Ariana (*ex horto,* P.I. 197822)
	506	20	Chile: Bio Bio, Los Angeles (P.I. 203305)
	644	40	Spain: Córdoba
	616	20	Australia

for *M. arvensis* from eastern Poland and the Markova (1983: 510) count of $2n = 66$ for *M. arvensis* ssp. *austriaca* (Jacq.) Briq. from Bulgaria.

Gill & al. (1973) studied variation in the morphology, cytology, reproductive biology and essential oils of North American populations of *Mentha arvensis*. They showed that they differ from those in Europe in leaf and calyx characters, but the extremes of variation overlap, and they chose not to separate them taxonomically. The counts that they made on 48 populations from Canada and the United States all had $2n = 96$. Tucker (personal communication) is currently studying the essential oils, morphology and cytology of the North American native *Mentha* and has proposed that the plants are hybrids between European *M. arvensis* and *M. longifolia*. However, a taxonomic change at this time would be premature and we will recognize the differences at the varietal level. Our six accessions have $2n = 96$.

Löve & Löve (1982: 353) reported $2n = 72$ for *Mentha arvensis* subsp. *borealis* (Michx.) R. L. Taylor & MacBryde collected in Manitoba, Canada. It is possibile that the plants had been introduced from Europe. Taylor & Mulligan (1968: 102) included

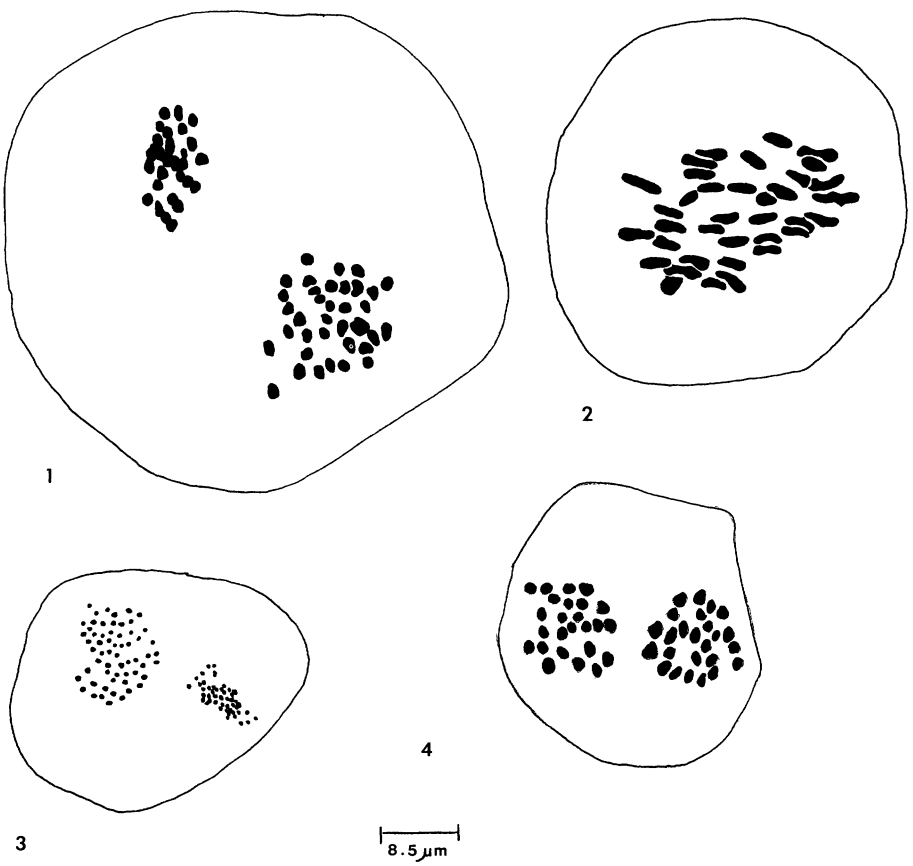

Fig. 1-4. Drawings of chromosomes in *Mentha* during meiosis in pollen mother cells. – 1, *M. australis*, M_{II}, $n = 36$; 2, *M. cunninghamii*, M_I, $2n = 36$ bivalents; 3, *M. diemenica*, M_{II}, $n = 60$; 4, *M. japonica*, M_{II}, $n = 25$.

two counts for *M. arvensis* from the Queen Charlotte Islands, $2n = 36$ and $n = 46$. The former, from a gravel pi, could have been introduced; for the latter, $n = 48$ is the expected count. Ikeda & al. (1970) found both 72 and 96 chromosome in a cytological survey of Japanese mint (*M. arvensis* var. *piperascens* Malinv. ex Holmes) growing wild in north-eastern Japan. They concluded that the plants with the lower chromosome number might be hybrids with *M. japonica*. Sokolovskaja & al. (1986) counted as $2n = 72$ on plants of *M. haplocalyx* Briq., an Asian segregate from the Soviet Far East.

In our studies, *Mentha longifolia* subsp. *longifolia* accessions from France, the Netherlands, and Uzbekistan were diploid ($2n = 24$) but two collections from Afghanistan were tetraploid ($2n = 48$). These counts are consistent with the findings of Harley & Brighton (1977). Gill (1984) reports $n = 10$ for *M. sylvestris* L., a synonym of *M. longifolia,* but this seems unlikely. Other diploid subspecies include two from South Africa (subsp. *polyadena* and subsp. *capensis* and one from the Himalayas (subsp. *hymalaiensis*). Astanova (1984) reports $2n = 18$ for *M. pamiroalaica* Boriss. from Tadjakistan, a taxon which is probably a segregate of *M. longifolia*. However, if that be the case, the count is unexpected.

The chromosome level of the morphologically diverse taxon *Mentha spicata* is variable (Schürhoff, 1929; Nagao, 1941). Some variants having distinct morphologies have been given taxonomic recognition, but for this report, the variation is informally categorized into rugose-leaf, glabrous-leaf, hairy-leaf and crisp-leaf forms. The rugose-leaf form, previously called *"M. cordifolia"* or, in the herb trade, cv. 'Kentucky Colonel', consistently has $2n = 48$. The glabrous-leaf plants can be either triploid ($2n = 36$) or tetraploid ($2n = 48$). The cultivar commercially grown in the United States, 'Native Spearmint', is triploid. The hairy-leaf and crisp-leaf forms that were counted were all tetraploid.

Accessions of *Mentha suaveolens* from Portugal, France, the Netherlands, and Bulgaria were counted and are all diploid ($2n = 24$). We also obtained similar counts for a variegated clone and subsp. *insularis,* which is native to the mountainous regions of the Balearic Islands, Corsica and Sardinia. We do not have the collection data for the latter for the latter accession; it was obtained from plants cultivated in France. Silvestre (1984) also reported $n = 12$ for *M. suaveolens* from Seville, Spain.

Most of the named hybrids in the genus are in *Mentha* sect. *Mentha*. Our counts of hybrid species include two of $2n = 24$ for *M.* ×*rotundifolia* and three of $2n = 36$ for *M.* ×*villosa* subsp. *alopecuroides*. These are the same numbers as were reported by Harley & Brighton (1977) and Gill (1981). Interspecific hybridization followed by vegetative propagation, and in some cases backcrossing to the parental species, has created much genotypic variation. Fagbemi (1975), Fagbemi & Morton (1982), Tucker & Fairbrothers (1990) and Tucker & al.(1991) made detailed studies of natural and artificial hybrids, using many techniques.

Mentha sect. *Preslia* ($x = 10, 12$). Our counts of $2n = 36$ in three accessions of *M. cervina* agrees with all previous reports for this taxon. The original placement of this species in a separate genus *Preslia*, by Opiz in 1824 (Harley, 1972a), was based on the presence of digitately lobed bracteoles and only four calyx teeth, features not found in other *Mentha* species. Harley (1972a), Harley & Brighton (1977), and Humphries & al. (1978) suggest that this species might be related to *M. gattefossei* (see below).

Mentha sect. *Pulegium* ($x = 10$, 12). The cytological picture of *M. gattefossei*, endemic to the Atlas Mountains of Morocco, remains confused. Early reports gave diploid numbers of 40 and 48 chromosomes. Harley & Brighton (1977) counted $2n =$ c. 32, and Humphries & al. (1978) reported $2n = 36$ in four collections from near Ifrane, Morocco. One of our collections has a diploid number of 40 and two are 48.

Recent counts of *Mentha pulegium* by Panetta (1986) and Chambers (1991) support the findings of Harley & Brighton (1977) and some of those of Morton (1956) which showed three ploidy levels ($2n = 20$, 30, and 40) on a base number of $x = 10$. The specimens upon which the counts were made represent a wide area of the species' range. However, Morton's report of $2n = 10$ for an accession of *M. pulegium* from the Liège Botanical Garden makes a base number of $x = 5$ a possibility. Harley & Brighton (1977) counted five accessions from England, Greece, Bulgaria and Morocco as $2n = 20$. A single accession from Portugal was $2n = 40$. Most of our counts from Europe and North Africa also are diploid ($2n = 20$). Plant material that originated from seed collected in the wild in Spain was tetraploid, and a plant from Poland was triploid ($2n = 30$). Our single count from Australia was diploid ($2n = 20$), as were earlier counts from three populations in Western Australia by Panetta (1986). Our Australian material was received from Hobart, Tasmania, but the collection site is unknown to us. The collections of *M. pulegium* from the United States were mostly from the Willamette Valley of Oregon where it is a weed of low pastures. Diploid, triploid and tetraploid populations were found within less than 30 km of each other. The single South American collection was diploid.

Conclusions

This work adds to a large base of chromosome data that has been accumulating since the first counts of *Mentha* species were published in 1929. We have expanded the geographical ranges from which counts of some common taxa have been made, and have added further information for some species with few or single previous counts: *M. australis, M. cunninghamii, M. diemenica* and *M. japonica.* But there are still taxa for which there are no chromosome counts: three from Australia, *M. laxiflora* Benth., *M. grandiflora* Benth. and *M. diemenica* var. *serpyllifolia* (Benth.) J. H. Willis. The lack of data prohibits a statement of base number(s) for *Mentha* sect. *Eriodontes.*

The NCGR collection lacks material of *Mentha micrantha* (Benth.) Des.-Shost., the only annual species in the genus. This taxon, placed in *M.* sect. *Pulegium,* grows in dry steppes and sink holes in south-east Russia and west Kazakhstan (Harley, 1972b; Borisova, 1977).

By reiterating the suggestions of Harley (1972a) and Humphries & al. (1978) on a possible relationship between *Mentha cervina, M. gattefossei* and *M. pulegium* we hope to stimulate new studies on the phylogeny of *M.* sect. *Preslia* and sect. *Pulegium.* We have observed plants of *M. pulegium, M. gattefossei* and *M. cervina* growning side by side, and the intermediate morphology of *M. gattefossei* is apparent. Maire (1922) suggested that *M. gattefossei* was ancestral to *M. cervina* but he did not speculate on its relationship with *M. pulegium* although all three species grow in North Africa. Harley (1972a) also suggests a close relationship between *M. gattefossei* and *M. cervina* based on their many morphological and ecological similarities.

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